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1 **Behavioural Responses of Broiler Chickens during Low Atmospheric Pressure Stunning**

2 Nikki Mackie and Dorothy E.F. McKeegan^{1*}

3 University of Bristol, Langford, Bristol, BS40 5DU, (nikki.mackie@bristol.ac.uk)

4 ¹Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary
5 and Life Sciences, University of Glasgow, Glasgow, UK, G61 1QH

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22 *Corresponding author

23 Dorothy.McKeegan@glasgow.ac.uk

24 Institute of Biodiversity, Animal Health and Comparative Medicine

25 University of Glasgow

26 Bearden Road

27 Glasgow

28 G61 1QH

29 UK

30

31 **Abstract**

32 Low atmospheric pressure stunning (LAPS) is a new irreversible stunning method for broiler chickens
33 (*Gallus gallus domesticus*), which has the potential to improve welfare during routine slaughter.
34 During LAPS, birds are placed in a hypobaric chamber that allows oxygen to be gradually removed
35 from the environment by the controlled removal of air; the staged process takes 280s and reaches final
36 decompression pressure that is 80.6 kPa below atmospheric pressure (nominally 101.3 kPa for an
37 absolute vacuum pressure of 20.7 kPa). In this study, the behaviour of broilers (50 individuals and 50
38 focal birds killed in groups of 20) was observed during LAPS. Latencies, total durations, single bout
39 durations and number of bouts were recorded for all behaviours. Three different decompression
40 curves were applied during the process (based on automatically applied settings related to ambient
41 temperature) and their effects on behaviour were investigated. Not all birds displayed all behaviours,
42 but a subset of behaviours (ataxia, loss of posture, clonic and tonic convulsions and leg paddling)
43 occurred in a consistent sequence. In individuals, mandibulation, headshaking and open bill breathing
44 occurred earliest at $44.5s \pm 31.6s$, $50.8s \pm 38.3s$ and $57.4s \pm 35.8s$ respectively after LAPS began.
45 Ataxia was observed on average at $57.3s \pm 11.5s$, with birds killed at colder temperatures taking
46 slightly longer to succumb to ataxia than those at warmer temperatures. Loss of posture (LOP) is
47 regarded as a behavioural marker for loss of consciousness and it occurred on average at $80.7s \pm$
48 $17.7s$. Clonic and tonic convulsions were displayed after LOP at $110.5s \pm 37.6s$ and $117.4s \pm 28.8s$
49 after LAPS onset respectively. Mean time to motionless was $199.4s \pm 21.3s$. The group data were
50 largely similar to that of individuals but were less reliable due to focal birds being obscured by
51 neighbours. Based on LOP, the data suggest that birds are in a conscious state for longer during
52 LAPS than in controlled atmosphere stunning with inert gases, but although the induction to
53 unconsciousness is more gradual, other behavioural responses were equivalent. The occurrence of
54 mandibulation, head shaking, and open bill breathing may be an indication of reduced welfare or may
55 be indications of a non-painful physiological responses to hypoxia in a hypobaric atmosphere. These
56 behaviours occurred at similar levels as seen in CAS with inert gases in poultry and the lack of escape

behaviours as well as absence of signs of severe dyspnoea suggest that LAPS is a humane approach to stunning of poultry.

Key words: Animal welfare, hypobaric hypoxia, humane slaughter, and loss of posture, low atmosphere pressure stunning

1. Introduction

Approximately 17.8 million broiler chickens (*Gallus gallus domesticus*) are killed in the UK every week (DEFRA, 2015), so welfare at the point of slaughter is an important issue. Electrical stunning is associated with various welfare concerns including shackling of conscious birds, pre-stun shocks and the risk of inadequate stunning (Raj, 2006). Recent EU legislation, Regulation (EC) no. 1099/2009 on the protection of animals at the time of killing (European Commission, 2009), provides stricter rules surrounding the use of electrical stunning which has fuelled increased uptake of controlled atmosphere stunning (CAS). While CAS has many welfare advantages (birds are not shackled while conscious; all birds are stunned), birds are not rendered unconscious immediately, which could potentially result in pain and suffering – if, for example, nociceptive concentrations of carbon dioxide were used (Raj, 2006; Shields and Raj, 2010). There has been much research on the welfare implications of CAS (reviewed in Raj, 2006) and most studies have focussed on identifying gas mixtures that result in the most humane stun. A related but novel approach, Low Atmospheric Pressure Stunning (LAPS), has been developed in the United States, in which birds are stunned by gradual decompression resulting in hypobaric hypoxia. Thus, during LAPS, air (and therefore oxygen) is gradually removed from the atmosphere, rendering the birds unconscious. LAPS is in routine commercial use at a poultry processing plant in Arkansas, having been given ‘no objection’ status by both the United States Department for Agriculture (USDA) in 2010 and the Canadian Food Inspection Agency in 2013.

Although rapid decompression is a source of welfare concern, it has been argued that gradual decompression can be humane (Vizzier-Thaxton et al., 2010). Previous research on LAPS identified

83 process variables for a suitably gradual decompression (Purswell et al., 2007), examined some aspects
84 of behaviour and corticosterone responses (Vizzier-Thaxton et al., 2010), meat quality (Battula et al.,
85 2008; Vizzier-Thaxton et al., 2010) and pathology (Vizzier-Thaxton et al., 2010). Other work
86 examining hypoxia in poultry leading to anoxia achieved with a gas environment has reported
87 favourable results for welfare (Woolley and Gentle, 1988; Raj et al., 1991), and supports the notion
88 that LAPS could be a welfare friendly approach. The evidence from pilots exposed to hypobaric
89 environments suggests that effects of slow decompression are specific to each individual and include
90 gradual loss of cognition and motor skills without conscious awareness of the loss of these functions
91 (Woodruff and Webb, 2011), though we note that care must be taken when making comparisons
92 between humans and birds given important differences in their anatomy and physiology. Available
93 evidence suggests that gradual hypoxia is promising as a humane method of stunning for poultry, but
94 more research is required.

95 McKeegan et al. (2013) examined electroencephalogram (EEG) and electrocardiogram (ECG)
96 responses of broilers undergoing LAPS using similar equipment and processes as used by Battula et
97 al. (2008) and Vizzier-Thaxton et al. (2010). Application of LAPS was associated with changes in the
98 EEG pattern in the form of highly significant increases in total power, decreases in mean frequency
99 and in particular, progressive increases in slow wave (delta) activity, indicating a gradual loss of
100 consciousness. ECG traces indicated an absence of heart rate elevation in the conscious period,
101 suggesting that birds do not find LAPS induction distressing. However, the study was limited to one
102 temperature range and 28 birds (due to the necessity of surgical implantation of EEG electrodes) and
103 behaviour was not observed, so the suggested time to loss of consciousness of 40s has not been
104 corroborated. Vizzier-Thaxton et al. (2010) incorporated some simple behavioural observations in
105 their study of LAPS, in which behaviours indicative of anoxia were seen, but only ten replications of
106 group observations were carried out. Detailed recordings of individual responses during LAPS is
107 required to provide important information on whether and to what extent gradual decompression is
108 associated with potentially negative behavioural responses. The primary objective of the study was to
109 carry out a detailed behavioural analysis of broiler chickens undergoing LAPS, both in groups and
110 individually, with a focus on behaviour occurring during induction to unconsciousness. The secondary

111 objectives were to investigate the effects of bird weight, and whether slightly adjusted decompression
112 settings (automatically applied in relation to ambient temperature) had any effect on behavioural
113 responses. Our aim was to create a timeline of behavioural events during LAPS and interpret this
114 with regards to its welfare implications and EU legal requirements for animals to be spared any
115 avoidable pain, distress or suffering during their killing and related operations.

116

117 **2. Methods**

118 *2.1. Subjects and Husbandry*

119 Fifty individuals and 50 groups of twenty commercial (mixed sex, as hatched) Ross 708 broiler
120 chickens (*Gallus gallus domesticus*) were observed undergoing LAPS in two experiments. In the
121 groups, one focal bird was observed in each LAPS run producing true replication. The birds were
122 randomly selected from a single flock by a catching crew at normal depopulation and then randomly
123 assigned to 50 groups of 21 (one of which was randomly selected to undergo LAPS individually).
124 Individual birds were killed at 49 days of age and group birds were killed the next day. Individual
125 birds were weighed and group weights were used to calculate means for birds subject to LAPS in
126 groups. Bird weights were as expected in the US commercial system; at the time of killing they
127 weighed 3.4 ± 0.5 kg (range 2.6-4.3 kg). The effects of gender could not be examined because the
128 birds were from a commercial flock and were not sexed. Before both experiments, the birds were
129 housed in 50 pens (1.22 x 1.22 m), either individually (with visual and auditory access to neighbours)
130 or in groups for 24 hours before the trials. The pens had wood shavings litter and access to water and
131 standard commercial diet. Before undergoing LAPS, the birds were feed restricted for eight hours and
132 water restricted for two hours to mimic commercial practice, in which birds would normally be
133 caught, transported and spend time in lairage without food and water. The last hour of each restriction
134 took place in a standard US transport container (2.44 x 1.22 m) (immediately before LAPS). The
135 trials were undertaken in Mississippi, USA, and therefore were not subject to UK legal requirements

136 through DEFRA or Home Office regulations. The experiments received ethical approval from the
137 Animal Welfare and Ethics Committee of the School of Veterinary Medicine, University of Glasgow.
138

139 2.2. LAPS Process

140 The LAPS chamber was developed by Technocatch in Mississippi, USA and is used commercially to
141 kill broilers for meat production. Technocatch has patented the system and the pressure curves applied
142 by the process. The chamber used in the current study is a research unit, but is identical to those used
143 commercially. The chamber is cylindrical (6.1-6.25 m in length and 2.13 m in diameter) and is
144 designed to accommodate two standard US transport containers. The required decompression curve is
145 automatically applied and controlled by a computer and once started, can only be stopped in the case
146 of an emergency. A variable airflow withdrawal process controlled by pumps alters the atmosphere
147 (Holloway, in prep). An infra-red camera (130° camera with 18 infra-red illuminators, Model #RVS-
148 507, RVS Systems) is fitted into every unit to observe the birds. A hydraulically operated door is
149 present that allows the entry of the transport containers and seals them into the chamber to begin the
150 process. The LAPS evacuation process takes exactly 280 seconds, after which the chamber is returned
151 to atmospheric pressure using a baffled air inlet, prior to the door opening and the exit of the transport
152 containers.

153 2.3. Temperature Settings

154 The temperature settings (pressure curves) are created automatically by a computer programme to
155 control the extraction of O₂ from the environment. Because cold air is denser and therefore contains
156 more oxygen than warm air and birds apparently respond differently to anoxia at different
157 temperatures, slightly different pressure reduction curves must be applied to achieve the same
158 hypobaric effect under different ambient conditions. As discussed by Holloway (in prep), water in the
159 LAPS chamber may also lead to modification of the rate of decompression based on temperature.
160 There are six temperature settings that are applied in accordance with ambient temperature and
161 temperature settings 4, 3 and 2 were applied in this study; all the curves converge on a final pressure

162 of 20.7 kPa. The pressure curves of all temperature settings are identical until 67 s into LAPS; this is
163 to avoid variability in decompression rate in the early stage of the process, which may have welfare
164 consequences. The aim of the temperature settings is to have all birds losing posture (and potentially
165 consciousness) at a consistent time. During the individual trials there were 11 birds in temperature
166 setting 2, temperature setting 3 was applied to 23 birds and setting 4 was applied to 16 birds. During
167 the group trial, each with 20 birds, 19 groups had temperature setting 2, 19 groups had temperature
168 setting 3 and 12 groups had temperature setting 4. Power calculations based on differences in
169 behaviour durations reported related studies on controlled atmosphere stunning (Abeyesinghe et al.
170 2007; Lambooij et al 1999 and Gerritzen et al 2004) revealed minimum sample size of 10 birds per
171 treatment group are required in order to achieve an actual power of 0.89. The temperature settings
172 were applied sequentially in accordance with ambient temperature change (setting 4 from 7-12 °C,
173 setting 3 from 13-18 °C, and setting 2 from 18-20 °C) throughout the trial days, resulting in an
174 unbalanced design.

175

176 *2.4. Trial Procedure*

177 Two different experiments were conducted for individuals and groups. In both, birds were placed in a
178 standard 5-tier US transport module (2.4 x 1.2 x 1.3 m; length x width x height with the second tier
179 from the top being used in the experiment (tier dimensions 1.12m x 1.14m x 0.25m; length x width x
180 height). In the individual trials, the tier was modified by reducing its area by 60% with a soft
181 polystyrene divider (1.12m x 0.36m x 0.25m; length x width x height). This was to minimise damage
182 to the bird when convulsing and to prevent the bird disappearing out of view during LAPS. In the
183 group trials, groups of twenty birds were placed in the allocated tier, without the divider. One hour
184 before the beginning of the trial the birds were transferred to the transport container to mimic lairage.
185 On entering the chamber, birds were filmed for 20s before the LAPS cycle started to determine
186 whether transfer to the LAPS chamber without decompression had an effect on the behaviour of the
187 birds. The focal bird in the group trials was chosen based on proximity to the camera. The trials took
188 place in March, when the temperature in Mississippi varied throughout the day, ambient temperatures

189 ranged from 9°C-20°C, and temperature settings 2, 3 and 4 were applied. During the trials, the birds
190 were watched in real time on a monitor to check for unexpected behaviour so that the run could be
191 aborted if necessary.

192

193 *2.5 Behavioural observations*

194 Detailed preliminary observations were carried out to define the ethogram and train the observer
195 before quantitative observations began. Table 1 shows the behaviours that were recorded during
196 LAPS. Description of behaviour categories was adapted from previous work on CAS (Lambooi et al.,
197 1999; Webster and Fletcher, 2001; Gerritzen et al., 2004; Abeyesinghe et al., 2007; Gerritzen, 2007;
198 McKeegan et al., 2007a; McKeegan et al., 2007b; Coenen et al., 2009). Observer XT (Version 12
199 basic package, live video watching: Noldus Information Technology, Wageningen, the Netherlands)
200 was used to record and analyse the behaviour variables (latencies, bouts and counts) before
201 transferring the data into Excel and R for statistical analysis (R Core team 2014).

202

203 *2.5. Statistical Analysis*

204 Variables were created relating to the latencies, durations, bout numbers and bout durations (where
205 appropriate) of the behaviours shown in Table 1. Following testing for normality with the Anderson
206 Darling test, using the nortest R package version 1.0-2 (Gross and Ligges, 2012), and checking
207 normality with a histogram of the data, a one-way analysis of variance (ANOVA) or Kruskal Wallis
208 tests were carried out with temperature setting was applied as a factor. In individuals, correlations
209 between behavioural parameters and body weight were carried out using Pearson's correlation and
210 Spearman's rank correlation, using the pspearson test R package version 0.3-0 (Savicky, 2014).
211 Where temperature setting did not have an effect, data was pooled for further analysis, but if
212 temperature setting was significant then weight correlations were carried out within each temperature
213 setting. To compare results between individuals and groups, Mann-Whitney U tests and independent

two sample t-tests were used where appropriate. When comparing individuals and groups, if temperature setting had a significant effect, analysis was carried out within temperature setting.

3. Results

3.1. Individual observations

Behaviour in the 20 s before LAPS began was not formally analysed but was generally unremarkable, with the majority of birds sitting. In individuals, 13 birds were seen to exhibit behaviour in addition to sitting; eight birds exhibited some restless behaviour, four showed open bill breathing, four showed mandibulation and one showed headshaking in addition. In groups, nine birds exhibited some restless behaviour, two showed open bill breathing, five showed mandibulation and one showed headshaking. A consistent series of behavioural responses to LAPS were observed: ataxia, loss of posture, clonic and tonic convulsions and leg paddling. The behaviours observed and the proportion of birds carrying out those behaviours are summarised in Tables 2 and 3. Descriptive statistics in the text are mean \pm SD.

Ataxia was observed in all birds and the latency to ataxia was 57.3 ± 11.5 s (Table 2). As shown in Figure 1, temperature setting had a significant effect on the latency to ataxia ($P = 0.004$, One-way ANOVA, $F_{2,47} = 6.142$). At temperature setting 2, applied when ambient temperatures were warmest, ataxia was earlier than at settings 3 and 4. The mean duration of ataxia was 23.4 ± 16.2 s (Table 2). Bird weight was positively associated with the duration of ataxia ($n = 50$, $P = 0.015$, Spearman's Correlation, $\rho = 0.341$, Figure 2) and a significant correlation remained following removal of an outlier, suggesting that this correlation is not artefactual. Loss of posture was observed in all birds with a mean latency of 80.7 ± 17.7 s (Table 2). Slow wing flapping was observed in 41/50 birds, with a mean latency of 129.6 ± 45.7 s (Table 2). Temperature setting had a significant effect on latency to slow wing flapping ($P = 0.003$, One-way ANOVA, $F_{2,38} = 6.989$), which was increased at temperature setting 3 compared with settings 2 and 4 (Figure 1). The mean total time spent slow wing flapping

239 was 8.8 ± 4.4 s. The mean duration of each slow flapping bout was 4.7 ± 2.4 s and the number of
240 bouts ranged from 1-5 (Table 3).

241

242 Clonic convulsions occurred with a mean latency of 110.5 ± 37.6 s (Table 2) and mean duration of
243 11.4 ± 5.7 s. The number of clonic convulsion bouts (2.5 ± 1.4 , range 1-7; Table 3) was affected by
244 temperature setting ($P = 0.030$, Kruskal Wallis, $X^2 = 7.015$, $df = 2$), being higher at temperature
245 setting 2 and reducing a stepwise fashion (Figure 3). Tonic convulsions had a mean latency of 117.4
246 ± 28.8 s (Table 2). Time to onset of tonic convulsions was affected by temperature setting ($P = 0.026$,
247 One-way ANOVA, $F_{2,46} = 3.975$), where exposure to LAPS at temperature setting 3 induced tonic
248 convulsions faster than at settings 3 and 4 (Figure 1). Tonic convulsions had a mean bout length of
249 5.9 ± 4.3 s and total duration of 19.0 ± 11.7 s (Table 3). The number of bouts of tonic convulsion (3.9
250 ± 2.3) was significantly different between temperature settings ($P = 0.037$, Kruskal Wallis, $X^2 =$
251 6.575 , $df = 2$), with birds exposed to temperature setting 4 exhibiting fewer bouts than those at
252 settings 2 and 3 (Figure 3). Leg paddling was observed in 42/50 birds, with a mean latency of 161.2
253 ± 29.6 s (Table 2). Temperature setting affected the total duration of leg paddling ($P = 0.028$, One-
254 way ANOVA, $F_{2,39} = 3.935$, $df = 2$) with birds at temperature setting 2 spending less time leg
255 paddling than individuals at temperature settings 3 and 4, representing a stepwise trend (Table 2).
256 Leg paddling bout durations were also affected by temperature setting ($P = 0.019$, Kruskal Wallis, X^2
257 $= 7.960$, $df = 2$) in the same way. Becoming motionless was observed in 49/50 birds (because one
258 bird moved out of sight) with a mean latency of 199.4 ± 21.3 s (Table 2).

259

260 Headshaking was observed in 38/50 birds, with mean latency of 50.8 ± 38.3 s and a mean number of
261 3.3 ± 2.8 (range 1-11). Open bill breathing was observed in 37/50 birds with a latency ranging from
262 4.3-187.9 s and 2.4 ± 2.1 bouts per bird. Mandibulation was observed in 16/50 birds with a mean
263 latency of 44.5 ± 31.6 s and 2.1 ± 1.5 bouts per bird (range 1-5). Eighteen birds reacted with alerting
264 behaviour ('notice') at the onset of LAPS. Pecking the environment was observed in 11/50 birds with
265 a mean latency of 55.8 ± 12.5 s and 2.6 ± 2.4 pecks per bird. Jumping was observed in 12 birds with a
266 mean latency of 112.8 ± 40.1 s and 1.7 ± 0.9 jumps per bird (range of 1-3). Four birds jumped before

267 loss of posture; three birds jumped once and one jumped a total of three times. The mean time to loss
268 of jaw tension was 103.8 ± 34.4 s. The latter behaviours were too rare to analyse in relation to weight
269 and temperature.

270

271 *3.2. Group observations*

272 The series of behavioural responses observed in groups was the same as individuals, and these are
273 summarised in Tables 4 and 5. Accurate observation of a focal bird in a group of 20 was challenging;
274 on several occasions birds could not be seen temporarily because they moved behind other birds
275 (average total time out of view was 42.8 s (where either the wings, head and/or whole body was out of
276 view). Eleven focal birds went completely out of view (average duration 18.5 ± 15.7 s). Latency to
277 ataxia in groups had a mean of 58.3 ± 8.9 s (Table 4). The duration of ataxia could only be noted in
278 40 birds because of lack of loss of posture (9 birds) and ataxia data (1 bird) and was 21.9 ± 10.4 s.
279 Loss of posture was reliably established in 41/50 birds with a mean latency of 80.4 ± 11.1 s (range
280 50.0-117.8 s).

281

282 Mean latency to slow wing flap was 104.5 ± 28.5 s (Table 4) with a mean duration of 6.7 ± 4.4 s. The
283 mean total number of bouts of slow wing flapping was 2.4 ± 1.5 (range 1-6) (Table 5). Forty-three
284 birds exhibited clonic convulsions with a mean latency of 128.2 ± 38.3 s (Table 4). Temperature
285 setting affected latency to clonic convulsions ($P = 0.036$, Kruskal Wallis, $X^2 = 6.674$, $df = 2$), which
286 was increased at temperature setting 2, compared with settings 3 and 4 (Figure 4). Tonic convulsions
287 had an mean onset of 129.4 ± 35.7 s (Table 4) and lasted 10.1 ± 6.9 s with a 3.5 ± 2.0 of bouts per
288 bird (range 1-8) (Table 5). The number of bouts of tonic convulsions were higher at temperature
289 setting 2 followed by temperature setting 3 and then temperature setting 4 ($P = 0.026$, Kruskal Wallis,
290 $X^2 = 7.262$, $df = 2$) (Figure 5). Leg paddling had a mean latency of 162.0 ± 27.0 s and a mean duration
291 of 9.0 ± 5.4 s (Table 4). Becoming motionless was observed in 48 of the birds (the other two were out
292 of view) with an average onset of 207.5 ± 12.0 s (Table 4), and was affected by temperature setting (P
293 $= <0.001$, Kruskal Wallis, $X^2 = 15.184$, $df = 2$) motionless happened latest at setting 2, followed by
294 setting 3 and 4 (Figure 4).

295

296 Headshaking was observed in 38/50 of focal birds in groups, with mean latency of 58.5 ± 29.6 s and
297 3.5 ± 2.9 times per bird (range 1-11). Open bill breathing was observed in 45/50 group birds with a
298 mean latency of 64.4 ± 29.3 s and more bouts per bird at temperature setting 2 compared to 3 and 4 (P
299 $= 0.009$, Kruskal Wallis, $X^2 = 9.337$, $df = 2$). Mandibulation was observed in 33/50 birds in groups
300 with a mean onset of 58.0 ± 43.7 s and mean number of bouts of 2.4 ± 2.7 . Twenty-three birds
301 showed 'notice' behaviour at the onset of LAPS. Only two individuals pecked the environment and
302 those that pecked did so only once. Fifteen birds jumped during LAPS, with the average jump
303 occurring 132.5 ± 39.1 s after LAPS onset. The mean number of jumping bouts was 2.1 ± 1.2 . Loss of
304 jaw tension was only observed in 4 birds with a latency of 95.7 ± 11.8 s, but we note that this
305 response was particularly difficult to observe in groups. The latter behaviours were too rare to analyse
306 in relation to weight and temperature.

307

308 *3.3. Comparisons between individuals and groups*

309 Some differences were noted between individuals and groups. However, these differences must be
310 interpreted with caution because some of the group data was not as reliable as the individual data due
311 to birds being frequently out of view. Latency to ataxia and slow wing flapping was shorter in
312 groups, while latency to headshake and show clonic convulsions were increased compared to
313 individuals (Table 6). Further, total duration and bout duration of slow wing flapping, clonic and
314 tonic convulsions were also shorter in groups than individuals, while the number of clonic convulsion
315 bouts and open bill breathing bouts were higher in groups (Table 6).

316 **4. Discussion**

317 This study provides the first comprehensive behavioural data for broilers undergoing LAPS, and a
318 consistent series of responses were observed. The data provide a basis for comparison with related
319 hypoxic killing methods such as CAS, and in general the same range of behaviour patterns was
320 apparent - ataxia, loss of posture, clonic and tonic convulsions, leg paddling and becoming

321 motionless. As has been noted in previous studies on CAS (e.g. Abeyesinghe et al., 2007), there were
322 qualitative and (to a greater extent) quantitative variations in behavioural responses to LAPS. These
323 differences were not accounted for by bodyweight (where analysis was possible) and presumably
324 relate to other factors which remain to be identified but could include physiological traits such as lung
325 capacity and air sac volume and response of the brain to anoxia. The individual variation seen in
326 broilers is analogous to the results of studies in man of response to hypobaric chambers during pilot
327 training which revealed a high degree of individual variation between the range of symptoms' and
328 signs experienced (Woodruff and Webb, 2011). The weight of the birds used in this study ranged
329 from 2.5 Kg to more than 4Kg, thus while the mean reflected larger US boiler weights, there was
330 some overlap with broilers weights usually seen in the Europe. Bird weight correlated with only one
331 behavioural variable (duration of ataxia), so it appears that bird weight has a minimal effect and this
332 concurs with commercial experience that bird size does not have a significant impact on the process.

333

334 Headshaking, mandibulation and open bill breathing (or other forms of respiratory disruption) have
335 been observed in many studies of poultry undergoing CAS with both hypercapnic and inert anoxic gas
336 mixtures and these were also seen with LAPS (in 76%, 32% and 74% of birds respectively).
337 Experiments involving exposure to anoxia with inert gases provide the most relevant comparisons to
338 LAPS (though note that in most cases CAS studies involve immersion in the gas and not gradual
339 replacement of air), and various authors have reported headshaking in response to Argon and Nitrogen
340 (Lambooi et al. 1999; Gerritzen et al., 2000; Webster and Fletcher 2001; McKeegan et al., 2007a;
341 Abeyesinghe et al., 2007). The mean number of headshakes observed in response to LAPS was 3, and
342 this is intermediate between previous reports of 0.5 and one for Argon and Nitrogen respectively
343 (McKeegan et al., 2007a) and nine for Argon (Gerritzen et al. 2000). Headshaking has been
344 interpreted as an aversive reaction to carbon dioxide (Raj, 1996) but this does not explain its
345 occurrence in response to inert gases. Headshaking may indicate disorientation, discomfort,
346 respiratory distress (Webster and Fletcher 2001) or arousal (Hughes 1983) but it was not seen in all
347 birds which we might expect if certain sensations causing headshaking were a direct consequence of
348 undergoing LAPS. There are concerns that expansion of gases in body tissues or sinuses may cause

349 discomfort or pain during LAPS. Future work with analgesic intervention could help to determine if
350 the headshaking seen in the early part of LAPS induction is pain related. Mandibulation was observed
351 in a minority of birds during LAPS; this behaviour has also been observed previously in response to
352 Argon and Nitrogen (Webster and Fletcher 2001; McKeegan et al 2007a) which suggests that the
353 reduction in oxygen or another environmental factor is stimulating gustatory or trigeminal receptors in
354 the mucosal membrane of the birds. The relevance of this behaviour to welfare is unclear; during
355 LAPS it may also serve the function of equalising pressure between the ears and oral cavity via the
356 Eustachian tubes.

357

358 Open bill breathing was recorded in three quarters of the birds and this may indicate some dyspnoea
359 (respiratory discomfort), similar to CAS. Open bill breathing has been interpreted as an indication of
360 breathlessness in birds (Gerritzen et al., 2004), and breathlessness in mammals was recently defined
361 as a negative affective experience relating to respiration with multiple qualities (Beausoleil and
362 Mellor, 2015). In humans, these include respiratory effort, air hunger (increased urge to breath) and
363 chest tightness (Beausoleil and Mellor, 2015), though it is not clear whether these all apply to birds,
364 which have a unique respiratory system of unidirectional air flow through the lungs and multiple air
365 sacs. It has been suggested that anoxia results in air hunger in humans (Moosavi et al., 2003), and
366 Beausoleil and Mellor (2015) suggest that this may have the greatest potential to compromise welfare
367 compared to other forms of respiratory discomfort. There is evidence that some dyspnoea occurs in
368 all CAS stunning mixtures that have been investigated, including Argon and Nitrogen (e.g. Gerritzen
369 et al., 2004; Abeyesinghe et al., 2007). During LAPS, birds exhibited an average of 2.4 bouts of open
370 bill breathing, which is very close to a previously reported value for exposure to Argon (2.25 bouts,
371 McKeegan et al., 2007a) but less than for a hypercapnic mixture in the same study (13 bouts). Given
372 that headshaking, mandibulation and open bill breathing are all seen during exposure to anoxic gases
373 (normobaric hypoxia) as well as during LAPS (hypobaric hypoxia); it is difficult to conclude whether
374 they are a response to hypoxia or decompression, or both. It is also difficult to determine if such signs
375 are part of the birds' normal physiological response to hypoxia or evidence of pain, for which there is

376 no direct indicator (EFSA, 2013). Indeed, a few birds exhibited mandibulation, head shaking and open
377 bill breathing before LAPS began.

378

379 Loss of posture has been widely interpreted as a proxy for loss of consciousness (Gerritzen et al.,
380 2004, EFSA 2013) and during LAPS loss of posture occurred on average at 80.7s in individually
381 killed birds and at 80.4s in group killed birds. In previous studies on CAS, immersion in inert anoxic
382 gases has tended to result in a much more rapid loss of posture (e.g. 15.6s in Argon, Lambooij et al.,
383 1999). The gradual nature of LAPS means that birds experience a longer induction and therefore
384 there is a greater time period where they could potentially experience negative welfare. However,
385 obvious escape behaviours that have been seen during CAS (e.g. McKeegan et al., 2007a) were not
386 seen during LAPS and previous work in which ECG data was collected during LAPS (McKeegan et
387 al., 2013), showed no evidence of heart rate increase during induction (albeit from an elevated
388 baseline). McKeegan et al (2013) suggested a time to loss of consciousness of 40s, based on spectral
389 analysis of EEG recordings during LAPS. This does not match the observations in the current study.
390 There are a number of possible explanations for this discrepancy; the most likely is that because the
391 birds undergo LAPS in complete darkness, the EEG response was confounded with sleep-like waves
392 that are induced by simulated eye-closure. Another factor to consider is that the EEG study took place
393 under different ambient conditions (high summer temperatures of 40 °C) and therefore a different
394 temperature setting and decompression curve was applied. Experiments recording EEG output and
395 behaviour within the same bird during LAPS are necessary to generate a corroborated time to loss of
396 consciousness on an individual bird basis.

397

398 Clonic convulsions and tonic convulsions are commonly seen in gas stunning (reviewed in Raj, 2006)
399 and were also seen in LAPS. During LAPS, convulsions always occurred after loss of posture,
400 indicating that birds are in an unconscious state (Gerritzen et al., 2004, EFSA 2013). A previous
401 behavioural study on LAPS determined that clonic wing flapping was a major cause of wing damage
402 in birds killed by LAPS (Vizzier-Thaxton et al., 2010) but this is not a welfare issue as the self-
403 inflicted injury occurs when the affected bird is unconscious. However, when birds are killed in

404 groups, as is done commercially, it may be possible for birds that are still in a conscious state to be
405 disturbed or even injured by other birds wing flapping. In the current study, the total duration of
406 clonic convulsions and slow wing flapping in groups was 14s which is similar to the 15.1s determined
407 by Vizzier-Thaxton et al (2010) who also made observations in groups. The wing flapping duration
408 was slightly higher for individually killed birds in the current study, with a 20.2s total duration. These
409 figures are very similar to those previously reported for anoxic CAS (17.5s in Argon and 15.7s in
410 Nitrogen, McKeegan et al 2007a).

411

412 Although placing birds individually in the LAPS chamber maximised visibility of their behavioural
413 responses, they are likely to have experienced some isolation stress (Cheng et al, 2003) which may
414 have affected the results. While most birds sat in a resting position before LAPS, some did show
415 restless behaviour (though this was observed in both groups and individuals). Applying LAPS to
416 groups of 20 birds was commercially relevant, but obscuration of the focal bird by neighbours made
417 observations difficult and the resulting data less accurate. Several significant differences between
418 individuals and focal birds in groups were found, but in almost every case these were in the same
419 direction – individuals had shorter latencies, longer durations and more bouts than groups. Increased
420 latencies are probably because in groups, some birds may have been out view the first time the
421 behaviour happened, and decreased durations/bouts are also likely to be due to behaviour sometimes
422 not being visible. Therefore, these differences are probably not meaningful and instead reflect the
423 limitations of accuracy of the group observations. Abeyesinghe et al (2007) reported differences in
424 responses to gas stunning mixtures between individuals and groups and concluded that these were at
425 least partially due to difficulties with observations in groups. In the current study, there were two
426 exceptions to the normal group effect; latency to show slow wing flapping was reduced in groups, and
427 this may be a genuine group effect, with wing flapping being caused by disturbance by neighbouring
428 birds. Focal birds in groups also showed more open bill breathing, but the reason for this is not clear
429 since the number of birds in the chamber should not significantly affect oxygen availability and other
430 measures relating to hypoxia such as time to loss of posture were not different between groups and

431 individuals. In the future, observing birds undergoing LAPS in small groups such as pairs or triplets
432 may be a good way to improve visibility while eliminating isolation stress.

433

434 The automatic temperature settings resulted in slightly different pressure curves being applied, but the
435 decompression rate in the first 67s of LAPS was never altered. In this study, only three of the six
436 available settings were applied (due to the limited ambient temperature range). While there were
437 some effects of temperature setting on behaviour, these were not consistent and many did not show
438 stepwise trends making their interpretation difficult. Time to ataxia did show a stepwise trend, being
439 greatest in colder conditions which may relate both to greater air density and lower humidity in cold
440 air (with consequently increased oxygen availability) and physiological responses to air temperature
441 affecting oxygen exchange. Conversely, time to motionless in groups was faster at colder
442 temperatures, which may indicate overcompensation in the decompression curve in the later part of
443 the LAPS cycle.

444

445 To be humane, stunning methods should produce insensibility with minimum welfare concerns
446 (Joseph et al., 2013). Like CAS, LAPS has many advantages for commercial poultry slaughter,
447 including avoiding live shackling and ensuring every bird is stunned. The behavioural data presented
448 here suggest that LAPS is largely equivalent to anoxic gas stunning in the range of behaviours it
449 elicits, except that due to the gradual nature of the decompression, birds take longer to lose
450 consciousness. In the conscious phase, birds exhibit behaviour which has been previously associated
451 with controlled atmosphere stunning, namely mandibulation, headshaking and open bill breathing, but
452 not more so than in CAS. This behavioural evidence suggests that LAPS is a humane method for
453 stunning poultry. Further work is required to understand the stimuli that give rise to behaviours that
454 may reflect reduced welfare and to corroborate behavioural indicators of time to loss of consciousness
455 with EEG measurements on an individual bird basis.

456

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560 Table 1. Behavioural categories recorded during LAPS

Behaviour	Description
Notice	Alert/restless movements of the head and/or restless movements of the body.
Mandibulation	Repetitive and rapid opening and closing of the bill.
Headshake	Rapid lateral head movement.
Open bill breathing	Breathing with bill open, with or without neck extension.
Ataxia	Apparent dizziness, staggering, swaying of body and/or head, attempts to stand/sit or flaps wings to try and regain balance.
Loss of posture	Unable to regain/maintain a controlled posture.
Clonic convulsion	Rapid/vigorous movement of the wings, a new bout was defined as following a pause of at least one second.
Tonic convulsion	Uncontrolled twitching (visible muscular spasms within the body). A new bout was defined as following a pause of at least one second.
Slow wing flapping	One short burst or prolonged slow/moderate movement of the wings, occurring without any twitching of the body. A new bout was defined by a pause of one second.
Leg paddling	Involuntary, usually alternating, leg movements in the air or towards the ground depending on the body position of the bird. Leg paddling can also be determined by an alternating upwards and downwards movement of the body if bird is lying sternal. A new bout was defined by a pause of one second. A new bout was defined by a pause of one second.
Loss of jaw tension	Bill open for more than 2s without deep breathing and/or neck extension.
Jump	Explosive movement from a sitting/lying position to stand and then immediately back to sitting/lying position.
Peck	Moving head backwards and forwards in a pecking motion.
Motionless	No discernible body or breathing movements.
Sitting	Legs underneath the body cavity and wings relaxed against body wall.
Standing	Standing with the body fully or partly lifted off of the ground.
Lying	Lying once posture is lost and not perceived to be purposefully controlling posture.
None/ not seen/ unsighted	No noticeable body movements, wing movements, leg movements or the bird was completely out of view.

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572 Table 2. Summary of behavioural results from the individual trials, showing the percentage
 573 of birds exhibiting each behaviour, and mean latency (Lat) and range, mean total duration
 (TD) and range, and results of one way ANOVA/Kruskal Wallis analysis for the effect of
 temperature setting.

Behaviour	Birds (%)	Mean (\pm SD) Lat (s)	Range Lat (s)	P value Lat	Mean (\pm SD) TD (s)	Range TD (s)	P value TD
Ataxia	100	57.3 (11.5)	17.8-77.2	0.004	23.4 (16.2)	5.8-105.2	0.285
Loss of posture	100	80.7(17.7)	58.8-182.5	0.072	-	-	-
Clonic	98	110.5 (37.6)	63.3-208.2	0.955	11.4 (5.7)	1.3-25.5	0.965
Tonic	98	117.4 (28.8)	73.9-185.3	0.026	19.0 (11.7)	1.3-61.1	0.303
Slow wing flap	82	129.6 (45.7)	10.2-209.5	0.003	8.8 (4.4)	0.8-17.7	0.675
Leg paddling	84	161.2 (29.6)	110.3-220.7	0.202	10.1 (6.2)	0.7-26.7	0.028
Motionless	98	199.4 (21.3)	158.2-245.6	0.136	-	-	-
Headshaking	76	50.8 (38.3)	3.3-167.3	0.108	-	-	-
Open bill breathing	74	57.4 (35.8)	4.3-187.9	0.727	-	-	-
Mandibulation	32	44.5 (31.6)	4.4-137.5	0.863	-	-	-

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All degrees of freedom (df) = 2. N=50.

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589 Table 3. Summary of behavioural results from the individual trials, showing the percentage
of birds exhibiting each behaviour, and mean single bout duration (SBD) and range, mean
number of bouts and range, and results of one way ANOVA/Kruskal Wallis analysis for
the effect of temperature setting.

Behaviour	Birds (%)	Mean (±SD) SBD (s)	Range SBD (s)	P value SBD	Mean (±SD) Number of Bouts	Range Number of Bouts	P value Number of Bouts
Clonic	98	5.5 (3.6)	1.3-19.3	0.707	2.5 (1.4)	1-7	0.030
Tonic	98	5.9 (4.3)	1.3-20.4	0.465	3.9 (2.3)	1-12	0.037
Slow wing flap	82	4.7 (2.4)	0.8-11.8	0.674	2.1 (1.1)	1-5	0.190
Leg paddling	84	7.5 (4.6)	0.7-19.7	0.019	1.5 (0.8)	1-3	0.795
Headshaking	76	-	-	-	3.3 (2.8)	1-11	0.827
Open bill breathing	74	-	-	-	2.4 (2.1)	1-10	0.750
Mandibulation	32	-	-	-	2.1 (1.5)	1-5	0.755

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All degrees of freedom (df) = 2. N=50

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Table 4. Summary of behavioural results from the group trials, showing the percentage of birds exhibiting each behaviour, and mean latency (Lat) and range, mean total duration (TD) and range, and results of one way ANOVA/Kruskal Wallis analysis for the effect of temperature setting.

Behaviour	Birds (%)	Mean (\pm SD) Lat (s)	Range Lat (s)	P value Lat	Mean (\pm SD) TD (s)	Range TD (s)	P value TD
Ataxia	98 / 80	58.3 (8.9)	39.6-78.6	0.520	21.9 (10.4)	4.5-45.0	0.781
Loss of posture	82	80.4 (11.1)	50.0-117.8	0.507	-	-	-
Clonic	86	128.2 (38.3)	66.7-204.6	0.036	7.3 (4.6)	1.0-21.2	0.191
Tonic	84	129.4 (35.7)	77.3-197.2	0.152	10.1 (6.9)	0.9-26.1	0.080
Slow wing flap	92	104.5 (28.5)	63.1-169.9	0.610	6.7 (4.4)	0.4-19.7	0.896
Leg paddling	62	162.0 (27.0)	104.5-207.4	0.228	9.0 (5.4)	1.1-19.1	0.921
Motionless	96	207.5 (12.0)	180.1-235.3	<0.001	-	-	-
Headshaking	76	58.5 (29.6)	4.1-147.8	0.461	-	-	-
Open bill breathing	90	64.4 (29.3)	5.4-162.3	0.380	-	-	-
Mandibulation	66	58.0 (43.7)	2.8-174.2	0.615	-	-	-

All degrees of freedom (df) = 2. N=50.

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Table 5. Summary of behavioural results from the group trials, showing the percentage of birds exhibiting each behaviour, and mean single bout duration (SBD) and range, mean number of bouts and range, and results of one way ANOVA/Kruskal Wallis analysis for the effect of temperature setting.

Behaviour	Birds (%)	Mean (±SD) SBD (s)	Range SBD (s)	P value SBD	Mean (±SD) Number of Bouts	Range Number of Bouts	P value Number of Bouts
Clonic	86	3.0 (1.6)	1.0-7.5	0.464	2.6 (1.6)	1-7	0.213
Tonic	84	2.8 (1.3)	0.9-6.6	0.839	3.5 (2.0)	1-8	0.026
Slow wing flap	92	3.1 (2.4)	0.4-12.6	0.129	2.4 (1.5)	1-6	0.305
Leg paddling	62	6.1 (3.9)	1.1 -17.2	0.220	1.6 (0.8)	1-4	0.212
Headshaking	76	-	-	-	3.5 (2.9)	1-11	0.971
Open bill breathing	90	-	-	-	3.2 (2.6)	1-15	0.009
Mandibulation	66	-	-	-	2.4 (2.7)	1-14	0.559

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All degrees of freedom (df) = 2. N=50.

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637 Table 6. Outcome of statistical comparisons between individual and group trials.

Behavioural response		Outcome of t-test/Mann Whitney U
Latency	Ataxia	$P = 0.003$, $t = -3.418$
	Slow wing flap	$P < 0.001$, $W = 260$
	Headshake	$P = 0.031$, $W = 514$
	Clonic convulsions	$P = 0.038$, $t = 2.257$
Total duration	Slow wing flap	$P = 0.025$, $W = 1206$
	Clonic convulsions	$P < 0.001$, $t = 3.856$
	Tonic convulsions	$P < 0.001$, $W = 1549$
Bout duration	Slow wing flap	$P < 0.001$, $W = 1388$
	Clonic convulsions	$P < 0.001$, $W = 1601.5$
	Tonic convulsions	$P < 0.001$, $W = 1576$
Number of bouts	Clonic convulsions	$P = 0.012$, $W = 145$
	Open bill breathing	$P = 0.028$, $W = 36$

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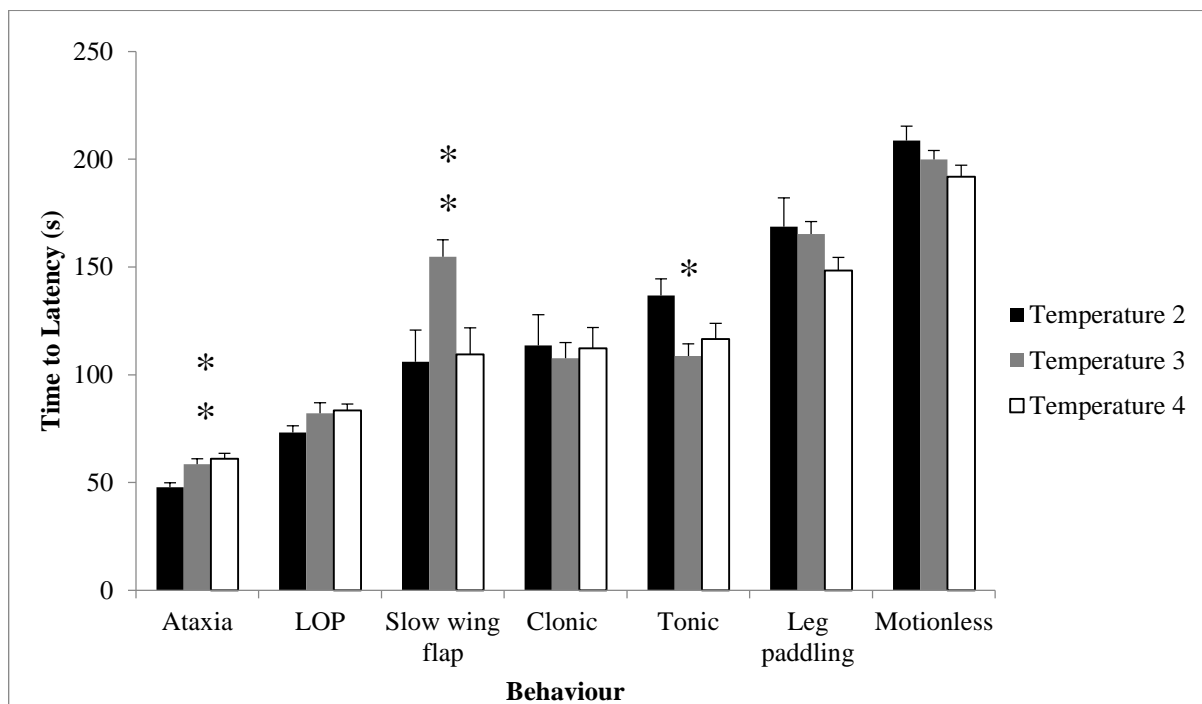
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Figure 1. Mean (SEM) time to latency of each behaviour at each temperature setting in individual killed birds. LOP = Loss of posture.
Temperature 2: 11 birds, Temperature 3: 23 birds, Temperature 4: 16 birds
 *= <0.05 , **= <0.01 , ***= <0.001

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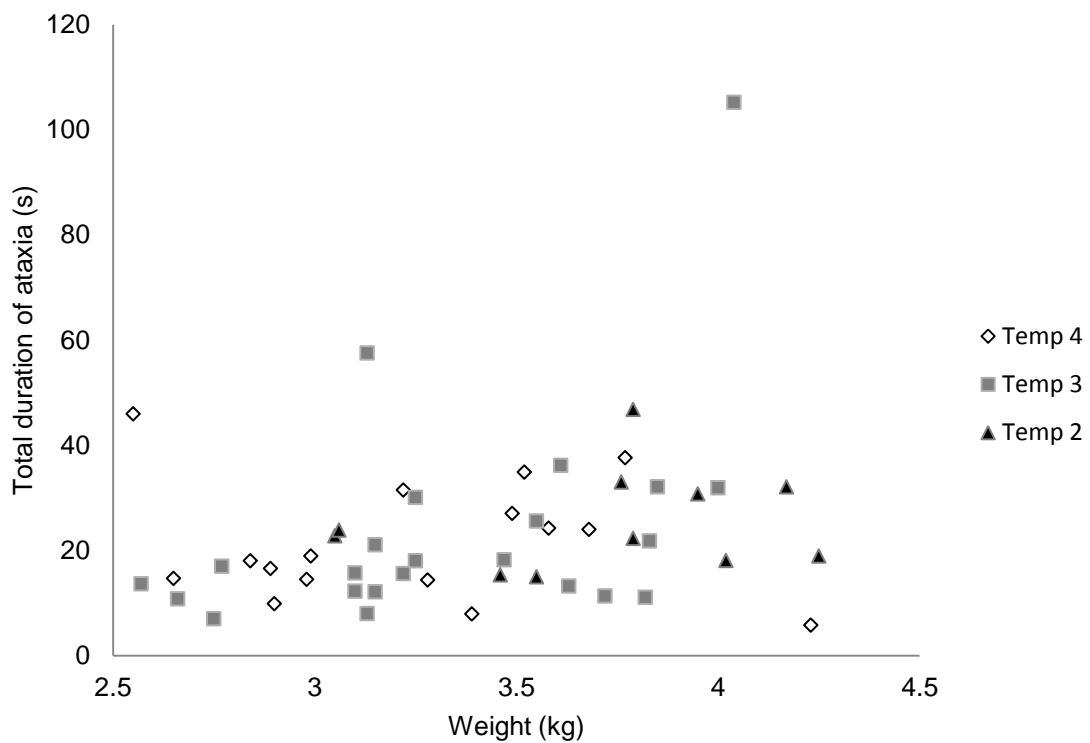
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662 Figure 2. Scatter plot showing the relationship between duration of ataxia and individual
663 bird body weight at each temperature setting; N=50.

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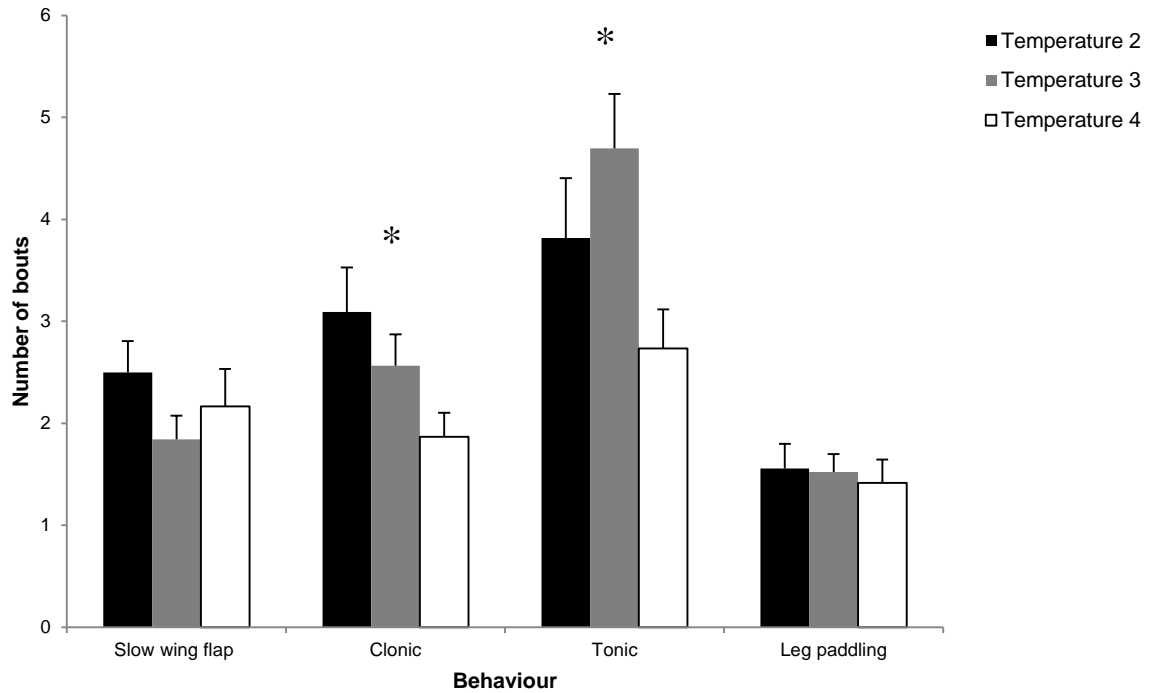
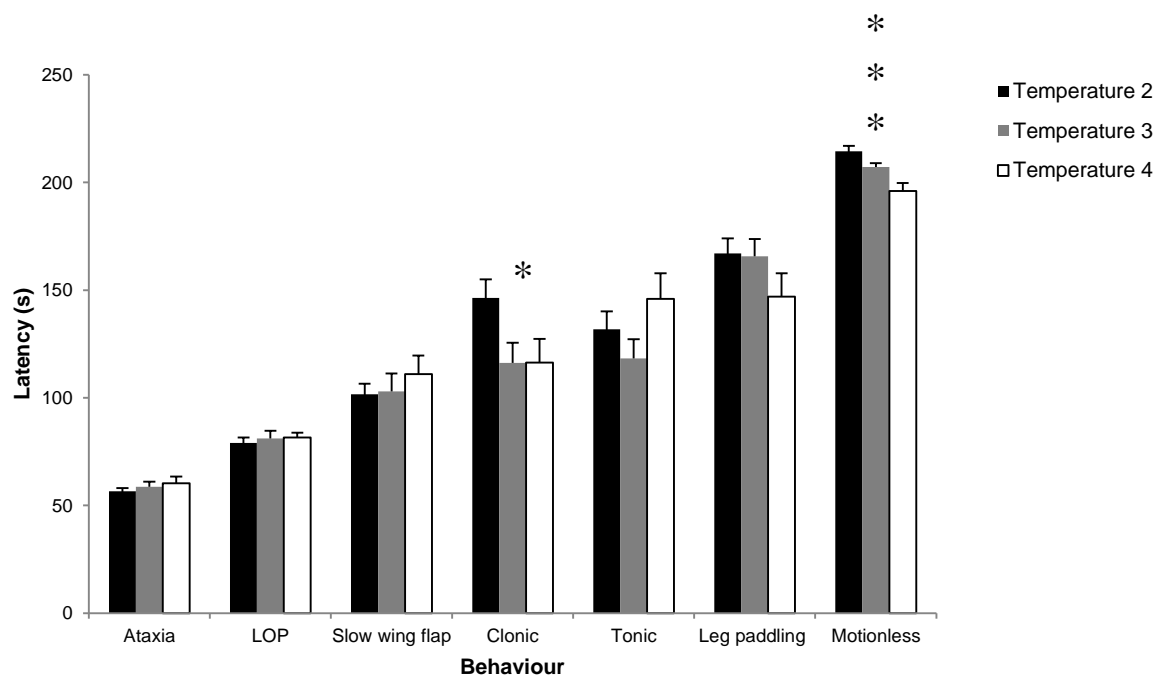


Figure 3. Mean (SEM) number of bouts of each behaviour at each temperature setting in individually killed birds.

Temperature setting 2: 11 birds, *Temperature setting 3:* 23 birds, *Temperature setting 4:* 16 birds

*= ≤ 0.05 , **= ≤ 0.01 , ***= ≤ 0.001

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Figure 4. Mean (SEM) latency of each behaviour at each temperature setting in group killed birds. LOP = Loss of posture.
Temperature setting 2: 19 birds, Temperature setting 3: 19 birds, Temperature setting 4: 12 birds
 *= <0.05 , **= <0.01 , ***= <0.001

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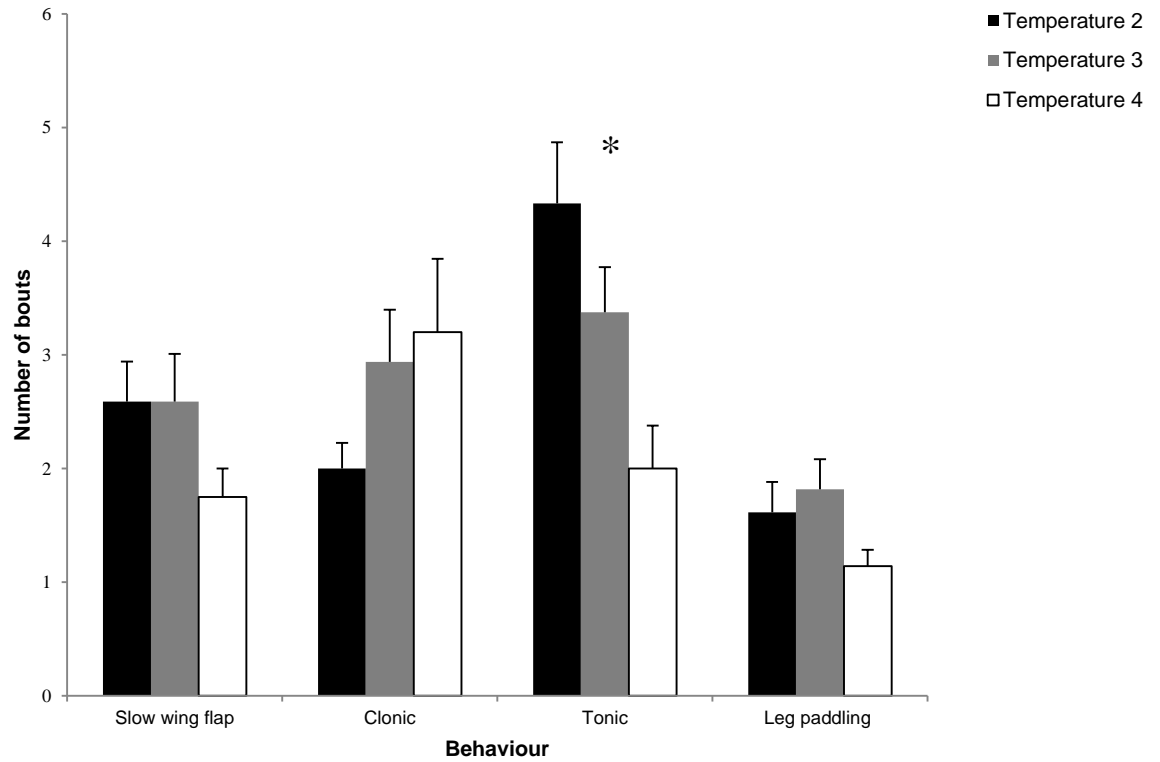


Figure 5. Mean (SEM) number of bouts of each behaviour at each temperature setting in group killed birds.
Temperature setting 2: 19 birds, Temperature setting 3: 19 birds, Temperature setting 4: 12 birds
 *= <0.05 , **= <0.01 , ***= <0.001